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# Trace elements in loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal: Bioaccumulation and tissue distribution

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## HIGHLIGHTS

- High levels of cadmium in loggerheads stranded in mainland Portugal.
- Evidence of bioaccumulation of renal cadmium.
- Cadmium-Zinc correlations were observed in liver and kidney tissues.
- The 3 largest loggerheads showed lower Cd concentrations than smaller turtles.

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## ABSTRACT

Pollution is among the most significant threats that endanger sea turtles worldwide. Waters off the Portuguese mainland are acknowledged as important feeding grounds for juvenile loggerheads. However, there is no data on trace element concentrations in marine turtles occurring in these waters. We present the first assessment of trace element concentrations in loggerhead turtles (*Caretta caretta*) occurring off the coast of mainland Portugal. Also, we compare our results with those from other areas and discuss parameters that may affect element concentrations. Trace element concentrations (As, Cd, Cu, Pb, Mn, Hg, Ni, Se, Zn) were determined in kidney, liver and muscle samples from 38 loggerheads stranded between 2011 and 2013. As was the only element with higher concentrations in muscle (14.78 $\mu g\ g^{-1}$ ww) than in liver or kidney. Considering non-essential elements, Cd presented the highest concentrations in kidney (34.67 $\mu g\ g^{-1}$) and liver (5.03 $\mu g\ g^{-1}$). Only a weak positive link was found between renal Cd and turtle size. Inter-elemental correlations were observed in both liver and kidney tissues. Hepatic Hg values (0.30 ± 0.03 $\mu g\ g^{-1}$) were higher than values reported in loggerheads in the Canary Islands but lower than in Mediterranean loggerheads. Cd concentrations in the present study were only exceeded by values found in turtles from the Pacific. Although many endogenous and exogenous parameters related with complex life cycle changes and wide geographic range may influence trace element accumulation, the concentrations of Cd are probably related to the importance of crustaceans in loggerhead diet in the Portuguese coast.

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## 1. Introduction

Along with other large marine vertebrates, marine turtles are known as key animal groups acting as sentinels of environmental disturbances, reflecting natural and anthropogenic threats on a

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wider portion of the marine ecosystem (McAloose and Newton, 2009; Fossi et al., 2012). According to the IUCN Marine Turtle Specialist Group, the most significant threats that endanger sea turtles include direct take, coastal development, global warming, fisheries impacts, pathogens and pollution (Mast et al., 2005).

Evaluating the effects of anthropogenic factors is presently a top global research priority for marine turtle conservation, including the impacts of pollution on marine turtles (Hamann et al., 2010). In fact, pollution has already been associated to the decline of some sea turtle populations (Gordon et al., 1998; Godley et al., 1999; Sakai et al., 2000). Sublethal effects caused by several pollutants, including trace elements, include impaired reproductive success (Perrault et al., 2011) and also deterioration of the immune system and other physiological functions in marine turtles (Aguirre et al., 1994; Day et al., 2007; Komoroske et al., 2011; Camacho et al., 2013).

Several studies reported the concentrations of trace elements in loggerhead turtles (*Caretta caretta*) in different areas of its wide distribution range (e.g. Gordon et al., 1998; Caurant et al., 1999; Godley et al., 1999; Saeki et al., 2000; Sakai et al., 2000; Storelli et al., 1998, 2005; Storelli and Marcotrigiano, 2003; Franzellitti et al., 2004; Torrent et al., 2004; Day et al., 2005; Maffucci et al., 2005; Andreani et al., 2008; García-Fernández et al., 2009; Jerez et al., 2010; D'Ilio et al., 2011; Camacho et al., 2013; López-Castro et al., 2013). These studies revealed how several ecological and biological variables (age, sex, diet or migration areas) influence trace element concentrations in loggerhead turtles (Caurant et al., 1999; Jerez et al., 2010; López-Castro et al., 2013).

In mainland Portugal, the analysis of a relatively high number of stranded marine turtles revealed that loggerhead turtles (242 loggerheads stranded in the 2009–2013 period) clearly include the Portuguese mainland coast in their oceanic pathways (Nicolau et al., 2016). The loggerhead turtle is currently categorized as "Vulnerable" by the IUCN (Casale and Tucker, 2015). Considering the loggerhead turtles Regional Management Units (RMUs, Wallace et al., 2010), the Atlantic Northwest and the Mediterranean RMUs overlap along the Portuguese mainland coast. These subpopulations have recently been re-categorized by the IUCN as Least Concern (Ceriani and Meylan, 2015; Casale, 2015) although they represent Low Risk–High Threat units (Wallace et al., 2011), indicating the need to mitigate threats that could lead to abundance declining in the future.

Despite the extensive literature regarding the concentrations of contaminants in loggerhead turtles, there is currently no information on trace element concentrations in marine turtles occurring off the coast of mainland Portugal. This is particularly relevant considering that the southern Portuguese coastal region represents an important hotspot for the neritic loggerhead turtle juveniles (Nicolau et al., 2016), where they may be exposed to contaminant-enriched coastal waters (van Geen et al., 1991; Mil-Homens et al., 2014).

Considering the importance of pollution as a threat to sea turtles, the present study aims at providing the first assessment of trace element concentrations in loggerhead turtles stranded in the Portuguese mainland coast, and relate them to concentrations reported in loggerheads elsewhere in the world.

## 2. Materials and methods

### 2.1. Sample collection

Loggerhead turtles stranded in the Portuguese mainland coast are routinely investigated (for example, following an alert given by the Maritime Authority) by experienced personnel belonging to the Portuguese stranding network, coordinated by the Institute for Nature Conservation and Forests (ICNF) and the Portuguese Wildlife Society (SPVS). Detailed necropsies were performed, where biometric data and samples were collected according to standard protocols (Wyneken, 2001). Kidney, liver and muscle samples were collected from 38 loggerhead turtles stranded between 2011 and 2013 (Fig. 1). Samples of loggerhead turtles were stored in glass vials and frozen (−20 °C) for posterior trace element analysis.

Curved carapace length (CCL) was registered (n = 38; CCL mean = 50.1 cm; CCL SD = 9.2 cm; CCL range = 35.5–75.5) and used as a proxy for age. Considering the minimum size of nesting females for the western North Atlantic stocks (CCL = 87.2 cm; TEWG, 2009), all specimens were identified as juveniles or subadults. It is also generally accepted that loggerheads from the North Atlantic presenting a CCL between 46 and 64 cm transit from oceanic waters to neritic habitats (Bjorndal et al., 2000).

### 2.2. Analytical procedure

Approximately 100–150 mg (wet weight, ww) of kidney, liver and muscle of loggerhead turtles were digested in teflon vessels with 2 mL of $HNO_3$ and 1 mL of $H_2O_2$ (Merck, Suprapure). Acid digestion of the samples was performed in a drying oven at 90 °C, overnight (14 h). All materials used in the digestion process were

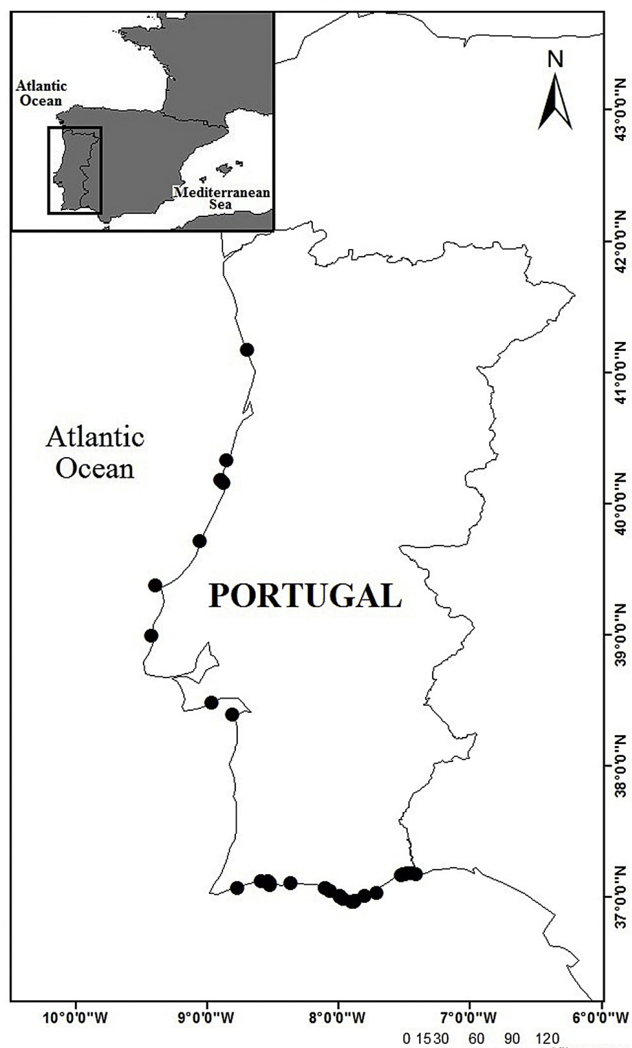


**Fig. 1.** Stranding locations of loggerhead sea turtles used in the present study.

**Table 1**
Trace element concentrations (mean ± SE, μg $g^{-1}$ ww) in different tissues of loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal. Concentrations are provided for the total number of animals and separately for oceanic juveniles (CCL < 46 cm), individuals transitioning from oceanic to neritic areas (CCL = 46–64 cm) and neritic subadults (CCL > 64 cm), according to Bjorndal et al. (2000).

| | Liver | | | | Kidney | | | | Muscle | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | <46 | 46–64 | >64 | Total | <46 | 46–64 | >64 | Total | <46 | 46–64 | >64 |
| Zn | 24.01 ± 0.94 | 23.85 ± 1.36 | 23.84 ± 1.28 | 25.88 ± 5.18 | 30.50 ± 1.49 | 30.35 ± 2.42 | 31.23 ± 2.10 | 25.49 ± 2.66 | 19.79 ± 0.82 | 18.62 ± 0.92 | 19.77 ± 1.19 | 24.64 ± 1.73 |
| Mn | 1.78 ± 0.09 | 1.99 ± 0.18 | 1.71 ± 0.12 | 1.39 ± 0.13 | 2.09 ± 0.14 | 2.26 ± 0.28 | 2.05 ± 0.18 | 1.70 ± 0.50 | 0.14 ± 0.01 | 0.14 ± 0.02 | 0.14 ± 0.01 | 0.12 ± 0.02 |
| Pb | 0.10 ± 0.01 | 0.08 ± 0.01 | 0.12 ± 0.02 | 0.08 ± 0.03 | 0.23 ± 0.03 | 0.22 ± 0.06 | 0.26 ± 0.04 | 0.11 ± 0.03 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.03 ± 0.02 |
| Cd | 5.03 ± 0.54 | 3.97 ± 0.50 | 5.99 ± 0.78 | 1.86 ± 0.37 | 34.67 ± 3.21 | 28.78 ± 4.37 | 41.06 ± 4.13 | 9.22 ± 0.72 | 0.16 ± 0.01 | 0.17 ± 0.02 | 0.18 ± 0.01 | 0.03 ± 0.00 |
| As | 4.49 ± 0.35 | 4.87 ± 0.69 | 4.13 ± 0.45 | 5.71 ± 0.49 | 6.28 ± 0.72 | 6.69 ± 1.52 | 5.61 ± 0.82 | 9.83 ± 2.52 | 14.78 ± 1.47 | 13.99 ± 2.46 | 14.68 ± 2.03 | 18.66 ± 4.15 |
| Cu | 5.99 ± 0.48 | 4.94 ± 0.71 | 6.70 ± 0.67 | 4.72 ± 0.24 | 1.73 ± 0.09 | 1.81 ± 0.13 | 1.75 ± 0.12 | 1.25 ± 0.24 | 0.55 ± 0.04 | 0.49 ± 0.06 | 0.58 ± 0.06 | 0.59 ± 0.03 |
| Hg | 0.30 ± 0.03 | 0.30 ± 0.03 | 0.30 ± 0.03 | 0.34 ± 0.22 | 0.21 ± 0.02 | 0.21 ± 0.03 | 0.21 ± 0.03 | 0.14 ± 0.09 | 0.05 ± 0.01 | 0.03 ± 0.01 | 0.06 ± 0.00 | 0.05 ± 0.04 |
| Ni | 0.14 ± 0.02 | 0.14 ± 0.03 | 0.14 ± 0.03 | 0.06 ± 0.01 | 0.44 ± 0.04 | 0.42 ± 0.04 | 0.48 ± 0.06 | 0.19 ± 0.08 | 0.08 ± 0.03 | 0.04 ± 0.01 | 0.10 ± 0.05 | 0.05 ± 0.03 |
| Se | 5.23 ± 0.20 | 5.07 ± 0.30 | 5.28 ± 0.26 | 5.46 ± 1.23 | 4.92 ± 0.29 | 4.87 ± 0.56 | 4.90 ± 0.37 | 5.35 ± 1.18 | 2.34 ± 0.09 | 2.24 ± 0.13 | 2.19 ± 0.11 | 3.06 ± 0.53 |

thoroughly acid-rinsed. After digestion, samples were diluted with ultrapure water and analyzed for nine trace elements [arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), zinc (Zn)], by ICP-MS (Perkin Elmer Elan 6000). To determine analytical accuracy, several blanks and standard reference material (*Squalus acanthias* - Dogfish liver (DOLT-3) and muscle (DORM-2)) (National Research Council, Canada) were prepared and analyzed along with samples. All of the analyzed trace elements exhibited concentrations above the detection limits of the analytical instruments, except for Hg in muscle samples of some individuals, which were considered missing values. Trace element concentrations are reported in μg $g^{-1}$, based on wet weight values (ww).

### 2.3. Statistical procedure

All data series were explored for outliers, collinearity, heterogeneity of variance and for visualization of potential relationships between response and explanatory variables, following Zuur et al. (2010).

Linear Models were used to determine the effect of curved carapace length (CCL) on the concentrations of trace elements in loggerhead turtles. Since the concentration values of each trace element were continuous, a Gaussian distribution was applied. Validation of the final model involved checking the assumptions of normality, homogeneity and independence of residuals, together with the lack of highly influential data points ("hat" values), through the use of plots involving residuals against fitted values and Q–Q plots, among others (Zuur et al., 2007). Assumptions of normality and homogeneity were also tested using the Shapiro–Wilk (Royston and Remark, 1995) and Breusch–Pagan (Breusch and Pagan, 1979) tests, respectively. Model validation decisions were based on both approaches. Turtles with CCL >64 cm (n = 3) were excluded from this analysis, due to the low number of samples in this size category.

In order to investigate the potential inter-elemental correlations, the Pearson correlation coefficient was used and the assumptions of normality and homogeneity of the residuals were checked, as described above for validation of the linear models.

Separate analyses were performed for each tissue type used in

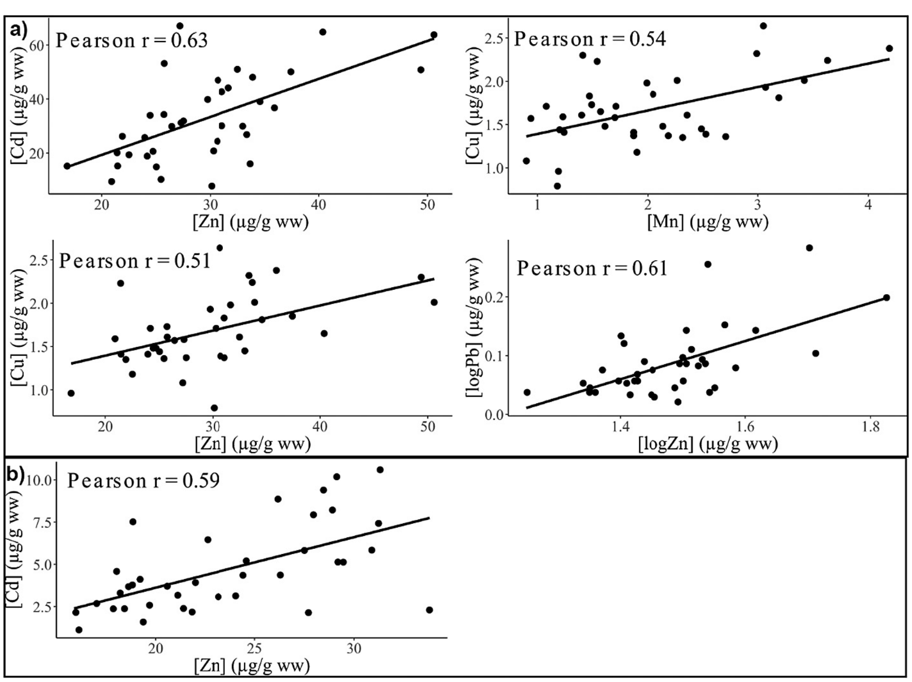


**Fig. 2.** Significant Pearson correlation coefficients (r) between several trace elements in kidney **(a)** and liver **(b)** of loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal. Only correlations yielding an r > 0.50 are shown.

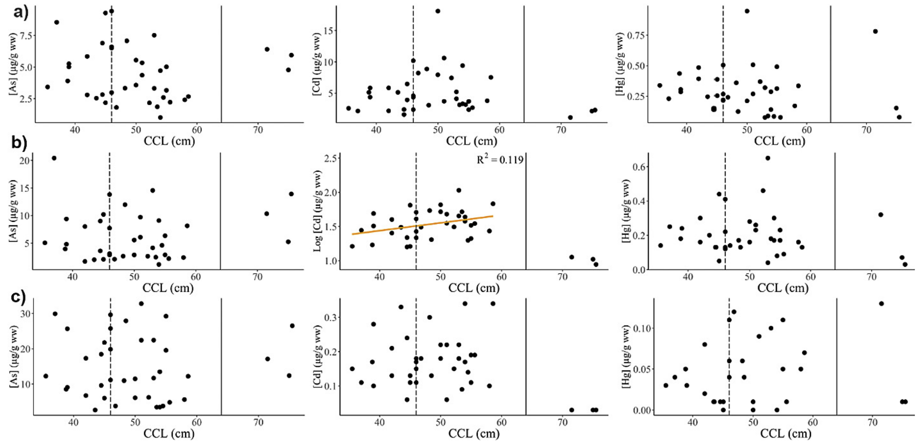


**Fig. 3.** Non-essential trace element concentrations in liver **(a)**, kidney **(b)** and muscle **(c)** of loggerhead turtles stranded in mainland Portugal, plotted against curved carapace length (CCL, in cm). Fitted regression line is included. The vertical dash and solid lines refer to the CCL limits for oceanic juveniles (CCL< 46 cm), individuals transitioning from oceanic to neritic areas (CCL = 46–64 cm) and neritic sub-adults (CCL> 64 cm), respectively, described by Bjorndal et al. (2000).

the present study. Statistical tests were performed in R v.3.2.3 (R Core Team, 2015).

## 3. Results

Trace element concentrations detected in the different loggerhead turtle tissues are presented in Table 1. Arsenic is the only element that shows higher concentrations in muscle (14.78 µg $g^{-1}$, SE = 1.47) compared to liver (4.49 µg $g^{-1}$, SE = 0.35) or kidney (6.28 µg $g^{-1}$, SE = 0.72) (Table 1). In turn, Cu, Hg and Se presented higher mean concentrations in hepatic samples, while Cd, Mn, Pb, Ni and Zn showed higher values in renal samples. Considering non-essential elements, Cd presented the highest concentrations in liver and kidney tissues (Table 1).

Several inter-elemental correlations were observed in both liver and kidney tissues of loggerhead turtles, as described in Fig. 2. In particular, a correlation between Cd and Zn was observed in both tissues. In renal samples, Zn concentrations also correlated with Pb and Cu, whereas Cu concentrations correlated with Mn concentrations. No strong correlations were observed in muscle samples.

Linear models revealed no relationship between any of the trace elements analyzed in the present study and turtle CCL, with the exception of a weak positive link between CCL and renal Cd (*t*-test = 2.109, p-value = 0.043, $r^2$ = 0.119). Fig. 3 shows turtle CCL plotted against the non-essential elements As, Cd and Hg and, in fact, no specific trends are detected except for renal Cd. Also, turtles with a CCL superior to 64 cm (excluded from linear models, see section describing the Statistical procedure) apparently show lower toxic element concentrations in comparison to smaller turtles, except for As.

## 4. Discussion

In general, the loggerhead turtles stranded in the Portuguese mainland presented trace element concentrations within the range of values reported in the Atlantic and Pacific oceans (Table 2). As for one of the most concerning toxic elements in the marine environment, Hg values in hepatic tissues obtained in the present study were lower than concentrations reported in loggerhead turtles from the acknowledged Hg-elevated Mediterranean basin (Storelli et al., 2005). However, Hg values in the present study were higher than values reported in loggerhead turtles in the Canary Islands (Torrent et al., 2004) (Table 2). Even though Hg levels in the analyzed tissues do not seem to be a cause of concern, according to Day et al. (2007) relatively low levels of Hg may affect health parameters in loggerhead sea turtles, including their immune function.

Relatively higher concentrations of renal Cd were also found in the present study (34.67 ± 3.21 µg $g^{-1}$ ww) compared to animals from the Atlantic coast of France and Spain (Caurant et al., 1999; Torrent et al., 2004), as well as the Adriatic sea and the Alboran sea (Storelli et al., 2005; García-Fernández et al., 2009). In fact, Cd concentrations in the present study were only exceeded by values found in turtles from the Pacific (Sakai et al., 1995; Gordon et al., 1998) (Table 2).

Knowledge about the actual toxic effects of many contaminants in higher marine vertebrates remains scarce and mostly based on values from laboratory experiments or surrogate species. Nonetheless, Cd is a non-essential element known to correlate with several health markers (Komoroske et al., 2011). Cd induces negative effects on essential elements metabolism and on endocrine and renal functions, being teratogenic and carcinogenic (Hopkins et al., 1999; Noel et al., 2004; Kitana and Callard, 2008; Ikonomopoulou et al., 2009; Simoniello et al., 2011). No toxicological data have been published specifically for marine turtles describing threshold concentrations of Cd above which detrimental effects would likely occur. Nevertheless, the renal concentrations of Cd found in the present study exceeded the toxic threshold previously suggested for evidence of Cd effects on vertebrates (10 µg $g^{-1}$ fresh weight, Eisler, 1985). It is noteworthy, however, that Torrent et al. (2004) reported seven turtles with Cd values between 20 and 60 µg $g^{-1}$ (ww) with no evidence of renal lesions.

Arsenic is the only element presenting higher concentrations in muscular tissue than in hepatic or renal tissues (Table 1). The highest As accumulation in muscle is in agreement with previous studies on loggerhead turtles (Saeki et al., 2000; Storelli et al., 1998; Storelli and Marcotrigiano, 2003). On the other hand, Torrent et al. (2004) revealed a higher As concentration in liver, where its toxicity could cause liver damage, and proposed that across their life cycle, changes in marine turtle feeding habitats and type of available prey in these habitats, may be responsible for the variation of As accumulation patterns.

Several marine vertebrates, including marine turtles, have developed detoxification strategies to mitigate the toxic effects of non-essential elements. For example, metallothioneins (MTs) are metal-binding proteins implicated in the detoxification of toxic trace elements such as Cd, Hg, Ag and Pb (Roesijadi, 1992; Das et al., 2000; Anan et al., 2002) and in the homeostasis of essential

**Table 2**
Mean trace element concentrations (μg $g^{-1}$ w.w.) of loggerhead turtles (*Caretta caretta*) available in literature.

| Ref. | Location | Liver | | | | | Kidney | | | | | Muscle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pb | Cd | As | Hg | Se | Pb | Cd | As | Hg | Se | Pb | Cd | As | Hg | Se |
| | **Atlantic** | | | | | | | | | | | | | | | |
| (1) | Portugal mainland | 0.10 | 5.03 | 4.49 | 0.30 | 5.23 | 0.23 | 34.67 | 6.28 | 0.21 | 4.92 | 0.01 | 0.16 | 14.78 | 0.05 | 2.34 |
| (2) | Canary Islands | 2.94 | 2.53 | 17.07 | 0.04 | | 2.44 | 5.01 | 13.80 | 0.04 | | 2.26 | 1.14 | 7.35 | | |
| (3) | Atlantic – France | | 2.58 | | | | | 13.3 | | | | | 0.08 | | | |
| (4) | South Carolina | | | | 0.594 | | | | | 0.214 | | | | | 0.155 | |
| (5) | Brasil south | | | | 0.74 | | | | | | | | | | 0,23 | |
| | **Mediterranean** | | | | | | | | | | | | | | | |
| (6) | Andalusia | 0.69 | 5.85 | | | | 0.17 | 10.49 | | | | 0.05 | 0.04 | | | |
| (7) | Adriatic + Ionian sea | 0.16 | 3.36 | | 0.43 | 3.54 | 0.12 | 8.35 | | 0.16 | 2.20 | 0.04 | 0.07 | | 0.18 | 1.65 |
| (8) | Adriatic (Italy) | | 2.84 | | | | | | | | | | 0.36 | | | |
| | **Pacific** | | | | | | | | | | | | | | | |
| (9) | Japan | | 9.29 | | 1.51 | | | 39.40 | | 0.25 | | | 0.06 | | 0.11 | |
| (10) | Queensland (Australia) | | 16.4 | 0.46 | 0.015 | 2.21 | | 28.30 | 0.71 | 0.045 | 1.52 | | | | | |

(1). Present study; (2). Torrent et al., 2004; (3). Caurant et al., 1999; (4). Day et al., 2005; (5). Soto et al., 2005; (6). García-Fernández et al., 2009; (7). Storelli et al., 2005; (8). Franzellitti et al., 2004; (9). Sakai et al., 1995; (10). Gordon et al., 1998.

elements (e.g. Cu, Zn, Roesijadi, 1992; Anan et al., 2002). Zinc is important in the regulation of MTs gene expression and, at least in mammals, MTs seem to occur more predominantly as ZnMT (reviewed in Sakulsak, 2012). A similar process in turtles might explain the strong correlations found in the present study between Zn-Cd, Zn-Pb and Zn-Cu. In fact, elements such as Cd, Pb and Cu show a higher binding affinity to MTs than Zn, being more capable of displacing Zn through a metal-metal exchange reaction, forming more stable MT complexes than other elements (Waalkes et al., 1984; Hamer, 1986; Sabolic et al., 2010; Sakulsak, 2012). Similar correlations have already been reported in other marine turtles (Sakai et al., 2000; Maffucci et al., 2005; Camacho et al., 2013), marine mammals (Méndez-Fernandez et al., 2014) and seabirds (Mendes et al., 2008; Ribeiro et al., 2009).

The concentrations of pollutants in loggerhead turtles may result from endogenous (e.g. sex, age) or exogenous factors (diet, prevailing environmental conditions), or from a combination of both (Aguilar et al., 1999; Caurant et al., 1999). Diet of loggerhead turtles stranded in mainland Portugal is mostly constituted by crustaceans (55.8% by number; 36.5% by weight) (Nicolau, 2016). Similar results were reported in other locations in the distribution range of the loggerhead Atlantic Northwest RMU, such as the West Atlantic nesting areas (Seney and Musick, 2007; Wallace et al., 2009). Crustaceans are known to be an important source of essential elements (e.g. Se and Zn), but also non-essential elements such as Cd (Turoczy et al., 2001; Nuñez-Nogueira and Rainbow, 2005; Karouna-Renier et al., 2007; Barrento et al., 2008; Reed et al., 2010; Maulvault et al., 2011). Therefore, the concentrations of Cd found in the present study may be associated to the dietary preferences of turtles in the Portuguese mainland coast.

A reduced importance of fish (recognized Hg vectors, e.g. Chouvelon et al., 2012) was reported in loggerhead turtle diet in Portuguese waters (Nicolau, 2016). The low fish intake may explain the lower Hg concentrations in the present study in comparison to other large vertebrate species in the same region (Mendes et al., 2008; Ribeiro et al., 2009; Ferreira et al., 2016; Monteiro et al., 2016a,b). Also, no Hg-Se correlation was detected in the present study. The non-critical concentrations of Hg were probably insufficient to trigger the formation of mercury and selenium complexes (HgSe) in hepatic tissues described as a detoxification Hg strategy in marine turtles and mammals (Storelli et al., 1998; Jerez et al., 2010; Frouin et al., 2012; Lailson-Brito et al., 2012).

The chronic exposure to non-essential trace elements in the marine environment associated to a limited capacity of excretion may lead to their bioaccumulation in long lived predators such as cetaceans (Ferreira et al., 2016; Monteiro et al., 2016a,b; 2017), seabirds (Mendes et al., 2008; Ribeiro et al., 2009) and turtles (Jerez et al., 2010; Komoroske et al., 2011). A positive, yet weak, link between renal Cd concentrations and turtle CCL was detected in the present study (excluding the three largest individuals from the sample, due to low sample size in that category). Considering the animals' CCL and previous stomach content analysis, which revealed the presence of both oceanic and neritic prey species (Nicolau, 2016), all animals in the present study were immature individuals inhabiting either oceanic waters (CCL < 46 cm) or a mix between oceanic and neritic animals (46 cm < CCL < 64 cm). Even the 3 largest loggerheads in our sample (CCL > 64 cm) were not yet mature individuals (considering the minimum size of nesting females for the western North Atlantic stocks; TEWG, 2009). Also, according to Mansfield and Putman (2013) both sub-adult and adult stage individuals probably undergo reversible oceanic-neritic transitions. As such, apart from the importance of crustaceans in the loggerhead turtle diet in the present study, the enhanced Cd concentrations may result from: 1) natural sources, due to the Cd enrichment in deeper waters due to biogeochemical processes (Bruland et al., 1978; Wu and Roshan, 2015), in the case of oceanic immature turtles, or to a lesser extent 2) anthropogenic sources, due to non-essential elements enrichment (e.g. Cd, Hg) in coastal waters owing to the proximity to contaminant sources (Delgado et al., 2011; Mil-Homens et al., 2014) in the case of transitioning turtles from oceanic to neritic habitats.

It is noteworthy that strong negative correlations were reported in previous studies between turtle CCL and non-essential elements such as Cd or Hg, which have been attributed to changes in physiological processes or in habitat use across life stages (Storelli et al., 1998; Sakai et al., 2000; Komoroske et al., 2011). In the present study, the 3 individuals with CCL >64 cm generally showed lower concentrations of most trace elements than the remaining animals. Some authors argued that the negative correlations between some trace elements and turtle CCL may be due to the ontogenetic shifts in dietary preferences associated with the transition from oceanic to neritic habitats (Sakai et al., 2000). If that was the case, higher concentrations of Hg and Cd would have been expected in larger turtles in the present study since those turtles ingest relatively more fish and cephalopods (Nicolau, 2016), which are known vectors of Hg and Cd, respectively (Bustamante et al., 1998; Chouvelon et al., 2012). Hence, the observed patterns in larger turtles may instead relate with trace elements biotransformation or elimination processes, including physiological aptitudes dependent on turtle maturity or exposure over time, such as up-regulation of metallothionein or dose-dependent assimilation changes (Davis and Cousins, 2000; Guirlet and Das, 2012; Sharma and Ebadi, 2014).

Apart from a baseline assessment of trace element concentrations in loggerheads in the study area, in particular, the present study revealed high levels of cadmium in loggerheads stranded in the Portuguese mainland. However, the effects of high cadmium levels on turtle physiological processes and their implications on turtle survival and reproduction remain unknown. Recently, Finlayson et al. (2016) stressed the need for further research on marine turtle ecotoxicology, particularly using non-invasive *in vitro* methods. In addition, considering the transoceanic range of loggerhead turtles, ecotoxicology studies should be accompanied by the assessment of diet variation and health markers across life cycle stages, from pelagic juveniles to neritic adults.

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